

Controlling Pecan Weevil with Beneficial Fungi: The Impact of Fungal Species and Fertilizer Regimes

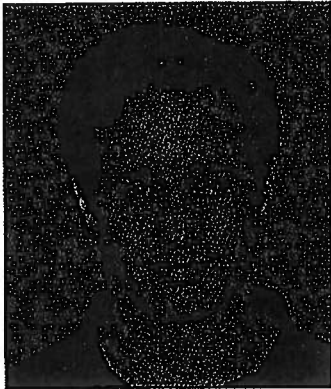
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Summary

The pecan weevil, *Curculio caryae* (Horn), is a key pest of pecan. Prior research indicated the potential for using beneficial fungi to suppress pecan weevil in the soil. We compared the efficacy of two fungal species, *Beauveria bassiana* (GHA strain) and *Metarhizium brunneum* (F52), in their ability to cause pecan weevil mortality. Results indicated that *B. bassiana* is superior to *M. brunneum* regardless of application method; consequently, the potential for applying *B. bassiana* to control pecan weevil was further explored. Specifically, the impact of different fertilizer regimes on the persistence of *B. bassiana* (GHA) in soil was determined. *B. bassiana* was applied to soil in a pecan orchard following one of several nitrogen-enhancement treatments—i.e., ammonium nitrate, crimson clover,

poultry litter, clover plus poultry litter, and a no-fertilizer control. Fertilizer treatments did not impact *B. bassiana* persistence. We conclude that standard fertilizers for nitrogen management, when applied according to recommended practices, are unlikely to negatively impact survival of *B. bassiana* when the fungus is applied for suppression of emerging pecan weevil (Shapiro-Ilan et al., 2013). Additional research on interactions between beneficial fungi and fertilizer amendments (or other tree nutrition or soil management practices) is merited.

Introduction

Pecan, [*Carya illinoensis* (Wangenh.) K. Koch], is an economically important North American nut crop

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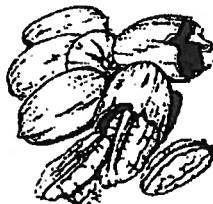
(Wood 2003). The pecan weevil, *Curculio caryae* (Horn), is a key pecan pest affecting orchard nutmeat yield and quality throughout the Southeastern US, and portions of Texas and Oklahoma. These insects have a two- or three-year life cycle with most adult weevils emerging from soil from late July through September to feed on and oviposit in, the cotyledons of developing fruit. Larval development is completed within the nutmeat of the ripening nut. Mature larvae then drop to the ground and burrow to a depth of 8-25 cm, form a soil cell, and over-winter. During the following autumn approximately 90% of larvae will pupate and spend the next nine months in the soil as adults (Harris 1985). The remaining 10% of the population spend about two years in the soil as larvae before pupating and emerge as adults in the third year (Harris 1985).

Current control recommendations for pecan weevil consist mainly of above-ground applications of chemical insecticides (e.g., carbaryl and certain pyrethroids) to suppress adults (Hudson et al. 2011). Application of chemical insecticides is recommended every 7-10 d during peak pecan weevil emergence (generally up to at least a six-week period) (Hudson et al. 2011). Due to problems associated with aphid and mite resurgence that often result

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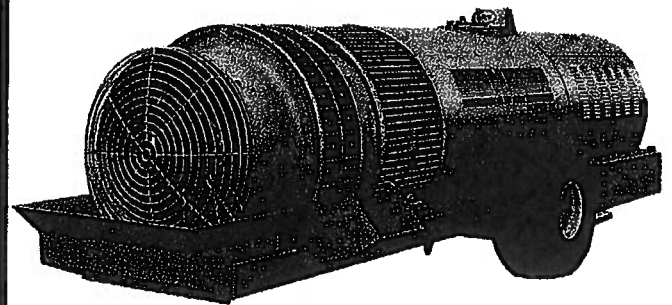
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from some chemicals used for weevil control (Dutcher and Payne 1985), as well as other environmental and regulatory concerns, research on developing alternative control strategies is warranted.

Beneficial fungi such as *Beauveria bassiana* and *Metarhizium* spp. constitute potential alternatives for pecan weevil control (Shapiro-Ilan 2003; Hudson et al., 2010). These fungi invade the insect host through the cuticle, replicate in the host hemocoel, and form external conidiophores to disperse their spores. These fungi can kill a variety of insects, including a number of weevil species and other beetle pests. Beneficial fungi can occur naturally in pecan orchards, in some cases providing up to 50% mortality in ground dwelling stages of pecan weevil (Shapiro-Ilan et al., 2008, 2012). Certain fungal species can also be applied to the orchard as bio-pesticides, thereby boosting the population of fungus and enhancing weevil suppression.

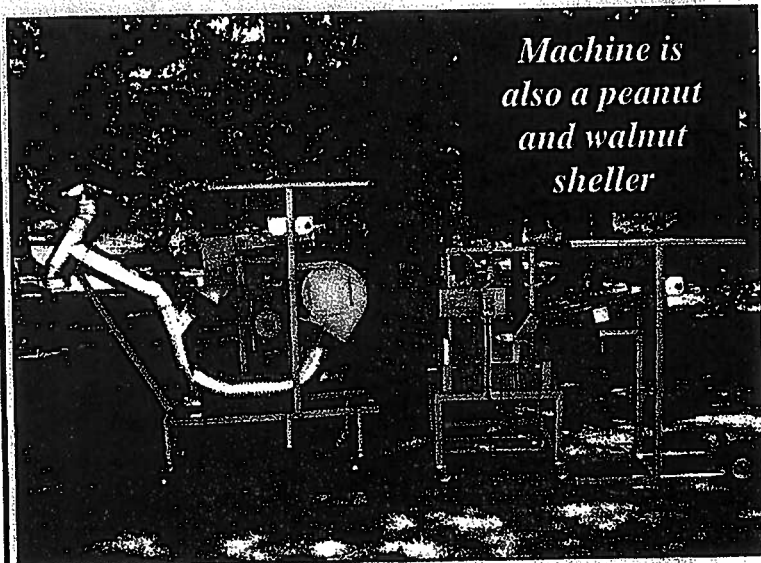
The most-studied beneficial entomopathogenic fungus for pecan weevil suppression is *B. bassiana* (Gottwald and Tedders 1983, Shapiro-Ilan et al. 2003, 2008, 2012). One approach to controlling pecan weevil is to expose adult insects to *B. bassiana* when they are emerging, i.e., before they enter the canopy and cause damage (Shapiro-Ilan et

al. 2008, Hudson et al. 2010). Shapiro-Ilan et al. (2008) demonstrated that the method of *B. bassiana* application can affect control of emerging pecan weevil adults (Shapiro-Ilan et al. 2008). Similar comparisons to determine the efficacy of different application methods for *M. brunneum*, and direct comparison to *B. bassiana*, have not been addressed; thus, our first objective was to compare efficacy of *B. bassiana* to *M. brunneum* for ability to cause pecan weevil mortality.

Certain abiotic soil parameters such as temperature, moisture, and the use of agrochemicals can affect survival and biocontrol efficacy of beneficial fungi. Fertilizer amendments may also impact persistence and efficacy of beneficial fungi, e.g., in laboratory studies Rosin et al. (1996, 1997) reported that composted cow manure enhanced persistence of *B. bassiana* whereas urea had no effect. Additionally, Shapiro-Ilan et al. (2012) reported that clover can enhance endemic (native) populations of *B. bassiana* in the orchard. Conceivably, differing fertilizer regimes in pecan management could affect post-application persistence or efficacy of commercially applied beneficial fungi, and thereby impact biocontrol of the pecan weevil. Therefore, the second objective of this study compared various fertilizer regimes for their impact on *B.*

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bassiana persistence. We chose to focus on *B. bassiana* for this objective because this fungus was found to be superior to *M. brunneum* in pest control efficacy in the first set of experiments.

Materials and Methods

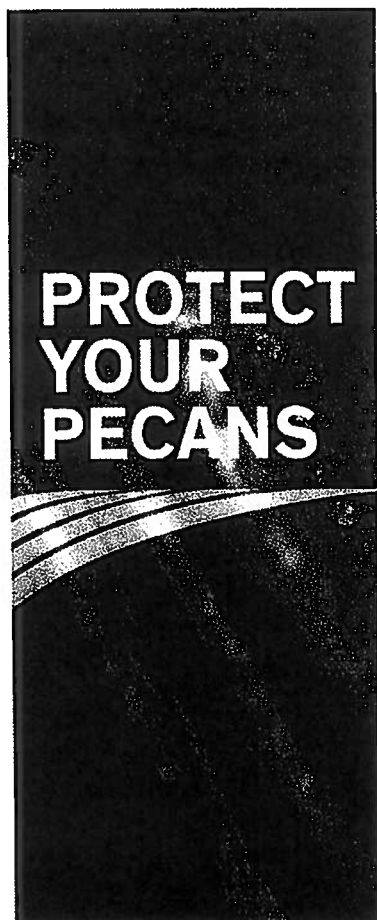
Objective I: Efficacy Comparison for Control of Pecan weevil. The effects of fungal species (*B. bassiana* vs. *M. brunneum*), and application methods for *M. brunneum*, on pecan weevil suppression was determined in field experiments in 2007 and 2008. The study was conducted in a pecan orchard at the USDA-ARS facility in Byron, GA. The orchard consisted of mature pecan trees (mixed 'Stuart' and 'Schley' varieties, approximately 70 years-old). Fungus application and assessment of treatment effects on pecan weevil suppression was based on methods described by Shapiro-Ilan et al. (2008, 2012). Treatments included *B. bassiana* applied to the trunk, and three methods of *M. brunneum* application, i.e., application to the trunk with or without SoyScreen as a potential UV protecting agents (Behle et al. 2009), and application to the soil surface. Commercially obtained *Beauveria bassiana* (GHA strain) was used in the study. This strain (GHA) has been labeled for use in controlling pecan weevil. *Metarhizium brunneum* (F52 strain) was also obtained

from a commercial company. An emulsifiable concentrate oil formulation was used for trunk applications, and a granular formulation (bulked with rice, approximately 5% active ingredient) was used for ground application. To determine treatment effects, adult pecan weevil were collected in Circle traps attached to pecan trunks. The weevils were taken to the laboratory and incubated at 25°C. After 14 days of incubation, the percentage of pecan weevil with mycosis (showing signs of fungal infection) and total mortality was recorded.

Objective II: Effects of fertilizers on persistence of *B. bassiana*. An experiment was conducted to determine the impact of different fertilizer regimes on *B. bassiana* persistence. We chose *B. bassiana* for this study because this fungus exhibited superior efficacy compared with *M. brunneum* in Objective I (see Results section) and therefore we decided to explore further factors that may affect *B. bassiana* performance. The study was conducted in a mixed cultivar pecan orchard at the University of Georgia, Tifton Campus, Ponder Research Farm near Tifton, GA. The orchard was managed under commercial conditions according to University of Georgia Cooperative Extension recommendations (Wells et al. 2007).

The following treatments were evaluated: 1) ammo-

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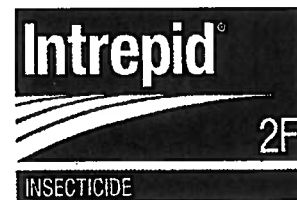
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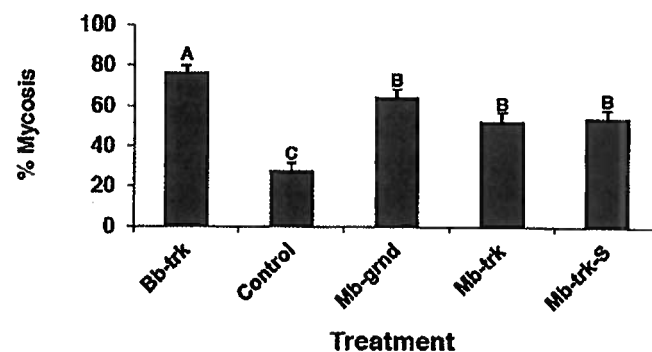
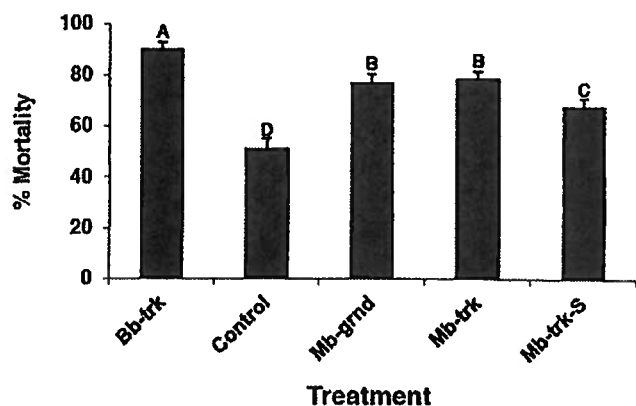


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nium nitrate; and 2) crimson clover; 3) poultry litter; 4) crimson clover + poultry litter; and 5) an untreated control (Wells 2011). Each plot consisted of a single pecan tree and its understory. Ammonium nitrate (NPK = 34-0-0) was applied at a rate of 84 kg N/ha on March 31, 2008, March 31, 2009, and March 29, 2010. This rate is considered a sufficient rate of N for southeastern pecan production (Wells et al. 2007). To ensure a full stand in the appropriate plots, crimson clover, was broadcast annually over the Bermuda grass sodded row middles beginning in October, 2007 at a rate of 33.6 kg/ha. Non-composted poultry litter was applied at a rate of 2240 kg per ha on the same dates that ammonium nitrate was added. Plots with clover or poultry litter did not receive N from other fertilizer sources. Individual plots received the same treatments in 2009 and 2010. The untreated control received no N fertilizer inputs.

Commercially produced *B. bassiana* (GHA strain; Botanigard®) was applied to all plots on July 22, 2009 and Fig. 1. Percentage mortality and mycosis of pecan weevils following application of *B. bassiana* (Bb) or *M. brunneum* (Mb) in a pecan orchard, 2007. Fungi were applied directly to the tree trunk (trk) or to the ground (grnd) under the tree canopy; trk-S indicates a trunk application with a SoyScreen adjuvant.



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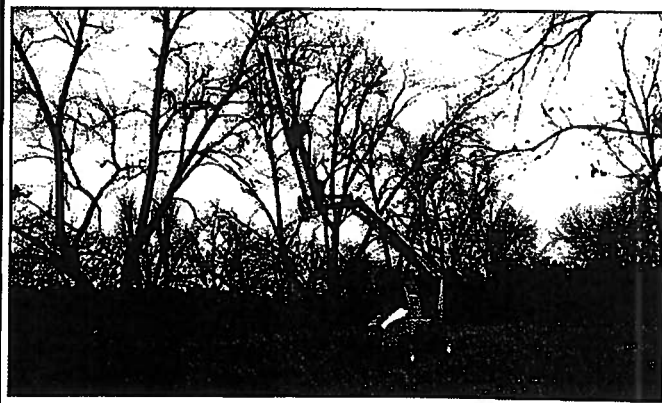
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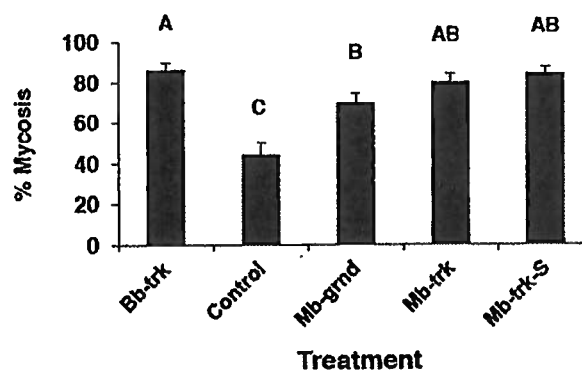
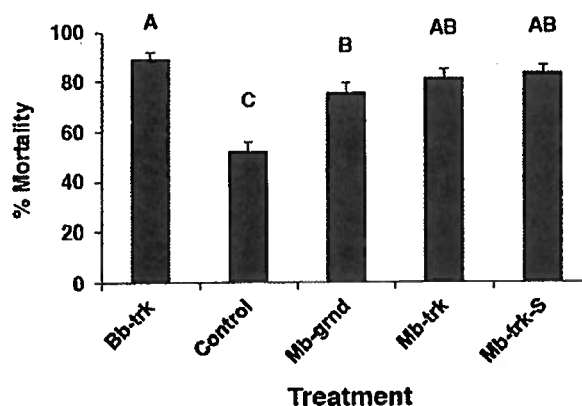


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July 28, 2010. Using watering cans, approximately 1.9×10^{12} conidia (fungal spores) were applied to the understory of each plot. The treatments were applied within a 3.1 m radius of the tree trunk. Fungal persistence was estimated using two methods: 1) by determining the number of colony-forming-units (CFUs = spores) per gram of soil and 2) baiting soil samples with a susceptible insect host. All experiments were properly replicated and treatment effects were determined using standard statistical analyses (Shapiro-Ilan et al. 2013).

Results

Objective I: Efficacy Comparison for Control of Pecan weevil. In 2007, all fungal treatments caused higher mortality and mycosis than was observed in the control, yet the *B. bassiana*-trunk treatment caused higher mortality and mycosis than all *M. brunneum* treatments (Fig. 1). In 2008, the *B. bassiana*-trunk treatment caused higher mortality and mycosis than the *M. brunneum* treatment applied to the ground, whereas the *M. brunneum* treatments Fig. 2. Percentage mortality and mycosis of pecan weevils following application of *B. bassiana* (Bb) or *M. brunneum* (Mb) in a pecan orchard, 2008. Fungi were applied directly to the tree trunk (trk) or to the ground (grnd) under the tree canopy; trk-S indicates a trunk application with a SoyScreen adjuvant.



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applied to the trunk were intermediate (Fig. 2).

Objective II: Effects of fertilizers on persistence of *B. bassiana*. In both years, no effects of fertilizer treatments on CFU (spore) counts were detected. Overall CFU counts per gram soil in 2009 ranged from 1652.38 to 4613.1 and from 94.72 to 539.44 in 2010. Similar to the assessment of CFUs, no fertilizer effects were detected in soil baiting data when analyzed over the experimental period.

Conclusions

- We observed superior efficacy in pecan weevil suppression using *B. bassiana* compared with several methods of *M. brunneum* application.

- Fertilizer treatments did not substantially impact *B. bassiana* persistence. Therefore, we conclude that fertilizers applied for nitrogen supplementation of orchard soils, under standard recommended practices for pecan, are unlikely to negatively impact survival of *B. bassiana* when the fungus is applied for pecan weevil suppression during weevil emergence.

- Conceivably, if the intensity of exposure to nitrogen fertilizers was increased, then impact to *B. bassiana* persistence would be more likely (and the result may be

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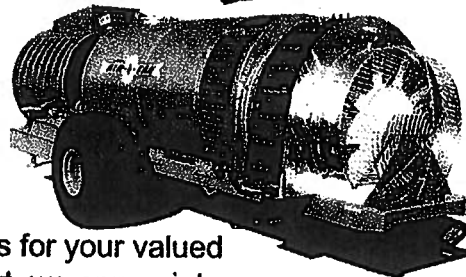
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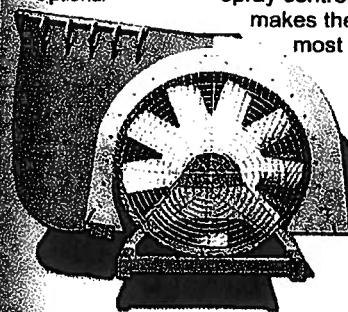


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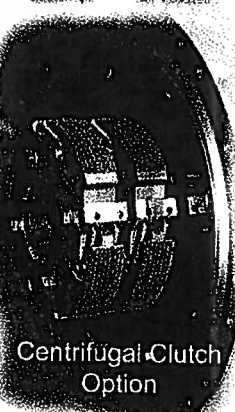
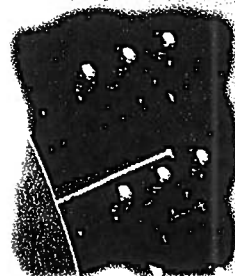


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positive or negative depending on the fertilizer).

- Additional research on interactions between beneficial fungi and fertilizer amendments (or other soil management practices) is merited.

Acknowledgments

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